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ABSTRACT

Dosage-mortality response of 4th-instar *Choristoneura fumiferana* to a microsporidian parasite, *Nosema fumiferanae*, was determined. Probit analysis produced an LD_{50} of 2.81×10^6 spores per larva with 95% lower and upper fiducial limits of 2.57×10^6 and 3.01×10^6 spores per larva, respectively. The mean number of days to 50% larval mortality was about 17 days. The greatest intensity of *N. fumiferanae* (20.7×10^6 spores per mg body weight) occurred after 5×10^6 spores were ingested.

RÉSUMÉ

On a déterminé, chez la larve du quatrième stade de *Choristoneura fumiferana*, le taux de mortalité en réaction aux doses d'une microsporidie *Nosema fumiferanae*. D'après l'analyses des probits, la DL_{50} est de $2,81 \times 10^6$ spores par larve, les limites inférieures et supérieures de confiance à 95% étant de 2,57 et de $3,01 \times 10^6$ spores par larve respectivement. Le nombre moyen de jours pour atteindre une mortalité larvaire de 50% était d'environ 17. Le taux maximal de *N. fumiferanae* ($20,7 \times 10^6$ spores par mg de poids corporel) a été observé après l'ingestion de 5×10^6 spores.

INTRODUCTION

Nosema fumiferanae is a naturally occurring microsporidian parasite of the spruce budworm, *Choristoneura fumiferana* (Thomson 1955). The spruce budworm is the most widely distributed destructive forest insect native to the North American continent and for this reason it is important that the host-parasite relationship is fully understood when considering use of the parasite in control procedures. One avenue of approach is the bioassay. In a bioassay the general procedure is to infect insects of a given age with a measured amount of the microbial agent and measure the effect such as mortality of the treated insects or days for this mortality to occur (Burges and Thomson 1971). If microbes are to be used in the control of pest insects, the dose that will provide the desired level of control must be determined.

A dosing technique for *N. fumiferanae* and its host the spruce budworm has been devised (Wilson 1983); however problems were encountered with loss of spores from treated needles, particularly with higher dosages. This paper reports the results obtained (increased efficiency of the parasite) when a sticker-spreader was used, thus producing a more accurate bioassay.

MATERIALS AND METHODS

Maintenance of insects, construction of the bioassay capsule and preparation of *N. fumiferanae* spores have been reported in detail (Wilson 1983). Disease-free 4th-instar larvae of the spruce budworm were used in all experiments. Tests were performed with these larvae under a regime of 16 h light and 8 h dark photo-period, 23 ± 1°C and a relative humidity of 60-80%. The bioassay capsule was assembled by placing a balsam fir *(Abies balsamea)* needle through a hole in the lid of a Beem embedding capsule. Spores of *N. fumiferanae* were

Table 1. The effects of various dosages of *Nosema fumiferanae* spores on morality, days to mortality and spore intensity, when spruce budworm ingested spores as 4th instar larvae.

Dose spores/ larva	Number in test	Percent larval mortality	Mean days to larval mortality ± S.D.	Percent pupal mortality	Mean spores/ mg tissue x 10^6 ± S.D.	Mean spores/ adult x 10^6 ± S.D.	
Control	52	6	-	0	-	-	-
5 x 10^5	78	9	23.0±10.7	5	11.8± 9.2	295±157	560±221
1 x 10^6	73	15	19.3± 4.5	2	14.9± 9.9	285±105	592±218
5 x 10^6	42	83	16.5± 8.4	0	20.7±23.7	-	-
1 x 10^7	46	91	14.2± 6.5	0	13.2±11.0	-	-
5 x 10^7	40	100	12.7± 4.6	0	9.9± 9.3	-	-

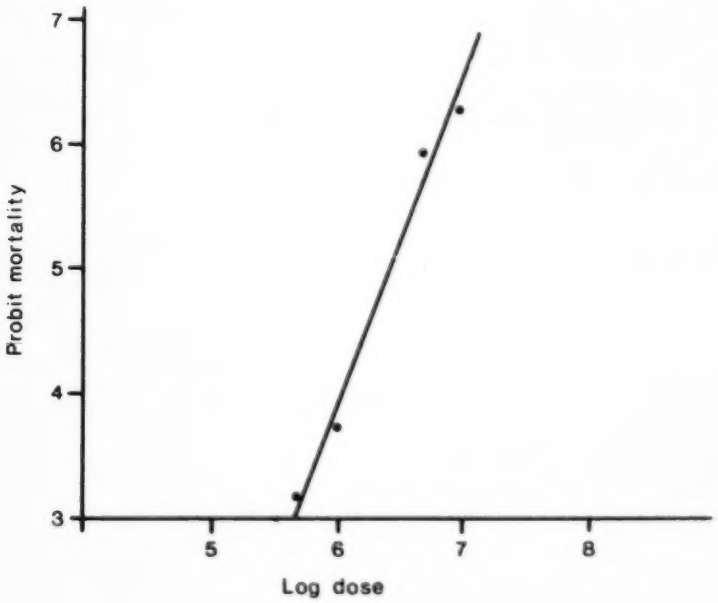

Fig. 1. Larval dosage-mortality response following ingestion of *Nosema fumiferana* spores by 4th-instar *Choristoneura fumiferana* larvae.

obtained from infected budworm reared in the laboratory and these spores were stored in distilled water at 5°C for up to 4 weeks before use.

Individual needles of balsam fir were treated with 5 µL of *N. fumiferanae* spore suspension, to give 5 x 10^5, 1 x 10^6, 5 x 10^6 and 1 x 10^7 spores per needle. The suspension contained 0.5% (V/V) of Nu-Film, a spreader-sticker. One larva was allowed to feed on one needle for 72 h, and those that consumed the entire treated area were returned to cups with artificial diet. All insects were reared individually. Control larvae were treated in a similar manner except that they were given 5 µL of distilled water containing 0.5% (V/V) Nu-Film. Experiments were replicated three times with 15-30 larvae per dose per replicate. Larval mortality, days to larval mortality, and infection intensity (number of microsporidian spores in each infected host) were recorded for each spore concentration. Spore counts, based on a method of Cantwell (1970), were performed on the insects after each was air-dried for a minimum of 7 days at about 32°C and weighed.

Results were analyzed using the F test for equality of means and the median lethal dosage (LD_{50}) was determined by probit analysis (Finney 1971).

RESULTS

Significant larval mortality (83%) occurred after ingestion of 5 x 10^6 spores, with 5 x 10^7 spores resulting in 100% mortality (Table 1). When the mortality probits were plotted over logarithm dosages, an infective dose-response regression line ($y = -12.34 + 2.69x$) with a standard error of 0.32 was obtained (Fig. 1). The LD_{50} value was 2.81 x 10^6 spore per larva with 95% lower and upper fiducial limits of 2.57 x 10^6 and 3.01 x 10^6 spores per larva, respectively.

The mean days to death with the various dosages tested are shown in Table 1 and Fig. 2A. As the dosage increased, days to death decreased. The range in mortality from the low-

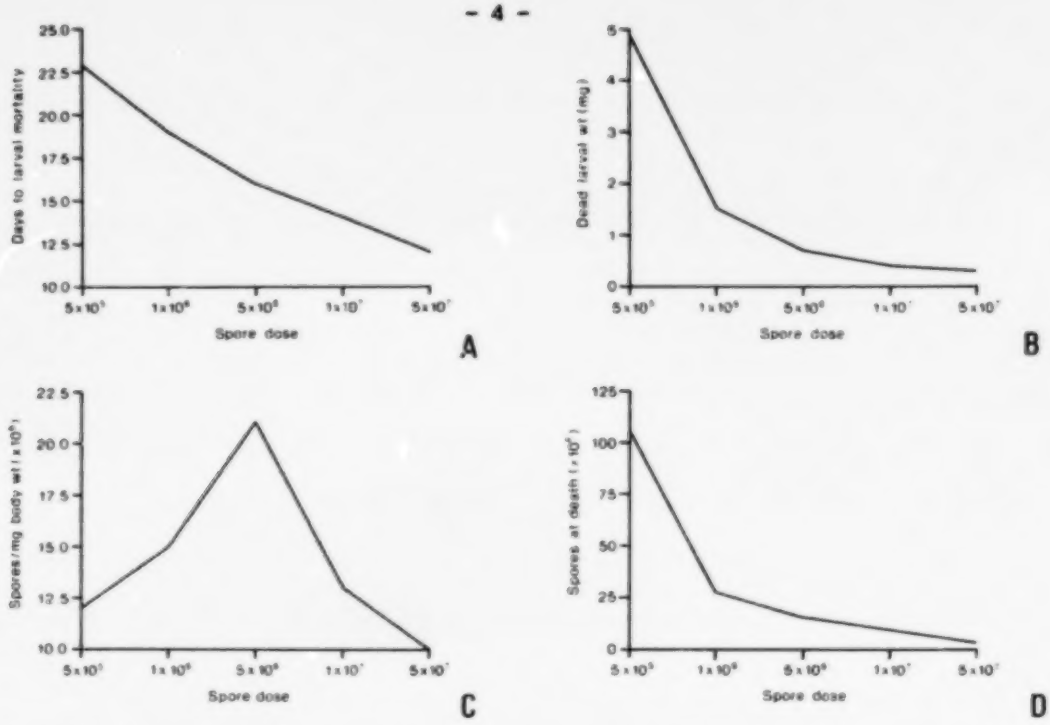

Fig. 2. The effects of various spore dosages of *N. fumiferanae* on: A, days to larval mortality; B, dead larval weight; C, spores per mg body weight; and D, spores per larva of *Choristoneura fumiferana* when treated as 4th-instar larvae.

est dosage (5 x 10^6 spores) to the highest (5 x 10^7 spores) was 23.0 ± 10.7 to 12.7 ± 4.6 days. The mean number of days to 50% larval mortality was about 17 days.

There was a substantial decrease in larval weights (air-dry basis) with increasing dosage, with the greatest decrease occurring from 5 x 10^5 to 5 x 10^6 spores per larva (Fig. 2B). These dosages produced a decrease from a mean of 4.8 to 0.65 mg. An analysis of variance indicated that the difference between the means was significant at the 1% level.

Figure 2C indicates the mean number of *N. fumiferanae* spores per mg of body weight (air-dry basis) after ingestion of various spore dosages. The greatest intensity of *N. fumiferanae* (20.7 x 10^6 spores per mg body weight) occurred after 5 x 10^6 spores were ingested. However, the standard deviation indicates considerable variation about the mean (Table 1). The actual mean spore count per larva at death decreased with increasing spore dosage (Fig. 2D). The spore count ranged from 106.9 x 10^6 to 2.8 x 10^6 spores per larva with a dosage of 5 x 10^5 to 5 x 10^7 spores.

DISCUSSION

The yield of spores from dead insects, and the number of spores functioning adults can harbor, is important when considering the role of a microsporidian parasite in nature. Also, if the parasite is going to be mass produced for field release, spore yield from infected larvae is important. In this test, spores per mg of tissue increased with dosage up to and including 5×10^6 spores; from this point spore production decreased, probably due to the shorter larval life of the insect with higher spore dosages. There is considerable variation in spore counts, and this has been reported for other insects. Hostounský and Weiser (1972) inoculated 4th-instar larvae of *Mamestra brassicae* by allowing caterpillars to drink different spore concentrations of *N. plodiae*. They also noted wide variation in spore counts of infected larvae when they died.

In general, the mortality of insects is related to the size of the initial spore dose of the microsporidia received. In previous tests with the spruce budworm, *N. fumiferanae* was applied to the host's food and it was assumed that the number of spores ingested was proportional to the dosage applied. In such a test Wilson (1974) indicated that the LD_{50} for 4th-instar larvae was about 2×10^7 spores per mL of suspension applied to balsam fir buds. In a more recent test, (Wilson 1983) spores were applied to individual balsam fir needles. Problems were encountered with loss of spores from the needles and results were similar to the test of 1974. However, with the use of a spreader-sticker, better adhesion of spores was obtained, and an LD_{50} of 2.8×10^6 spores per 4th-instar larva was realized. Many reports list the ID_{50} (Infective Dose) or IC_{50} (Infective Concentration) for various microsporidia and their hosts, but not an LD_{50}. One cannot assume that because an insect is infected it will die; in fact, Weiser (1976) indicates that in some of the reports he reviewed infective doses were usually not lethal. Reports indicate that a considerable range of virulence can occur in different *Nosema* species. For example, Kellen and Lindegren (1974) determined the dosage-mortality relationships for *Plodia interpunctella* and two microsporidia, *N. plodiae*

and *Nosema heterosporum* when fed to 10-day-old larvae. The LD_{50} for *N. plodiae* was 8.09 x 10^6 spores per g of diet and that for *N. heterosporum* 4.52 x 10^3 spores per g of diet. The LD_{50} for spruce budworm is closer to that of the less virulent *N. plodiae*. The authors suggest that the less virulent microsporidium could remain established in a population because of transovarial transmission and because nonlethal infections can occur. Thus repeated introductions in control programs would not be necessary. This scenario would also hold true for *N. fumiferanae* and the spruce budworm.

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